SYNOPSIS FOR REGISTRATION OF Ph.D

On

STABILITY INDICATING ANALYTICAL METHOD DEVELOPMENT FOR THE ESTIMATION OF ANTIHYPERTENSIVE DRUGS

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ABSTRACT:

The purpose of the method validation is to ensure that a selected analytical method will give reproducible and reliable result that are adequate for the intended purpose .The validation of analytical method will be done according to ICH Guideline.

Need for Analytical method development:

1) A systematic analytical method may not be available for the drug and combination in literature.

2) Reported analytical procedure may require expensive reagent and solvent.

3) Any official method not reported in IP,BP and USP for estimation of drug or combination.

INTRODUCTION:

Analytical chemistry

Analytical chemistry deals with methods for determining the chemical composition of samples of matter. A qualitative method yields information about the identity of atomic or molecular species or the functional groups in the sample; a quantitative method, in contrast, provides numerical information as to the relative amount of one or more of these components.

1. **Qualitative analysis –** Information regarding the presence or absence of one or more components of the sample.
2. **Quantitative analysis –** Information regarding the amount of components of the sample, however the required information is finally obtained by measuring some physical property that is characteristically related to the compound of interest.

Factors Affecting the Choice of Analytical Method

Analytical techniques have different degrees of sophistication, sensitivity and selectivity, as well as, different cost and time requirements. An important task for the analyst is to select best procedure for a given determination this will require careful consideration of the following criteria:

a) The type of analysis required: elemental or molecular, routine or occasional.

b) Problem arising from the nature of the material to be investigated, e.g. radio-active substance, corrosive substance, substances affected by water.

c) Possible interference from components of the material other than those of interest.

d) The concentration range to be investigated.

e) The accuracy required.

f) The facilities available, particularly the instrument.

g) The time required to complete the analysis.

h) The number of analysis of similar type which have to be performed.

Classification of Analytical Methods

The various methods of analysis can be broadly classified into two categories; Classical methods and Instrumental methods: -

1. Classical Methods

A. Volumetric Methods

In volumetric, also called titrimetric, procedures the volume or mass of a standard reagent required to react completely with the analyte was measured.

B. Gravimetric Methods:

In gravimetric measurements, the mass of the analyte or some compound produced from the analyte was determined. The extent of their general application is, however, decreasing with the passage of time and with the advent of instrumental methods to supplant them.

2. Instrumental Methods:

These methods are based upon the measurement of some physical properties as conductivity, electrode potential, light absorption or emission, mass-to-charge

ratio and fluorescence of substance. There are many techniques available for the analysis of analytes:

a) Spectroscopic Analysis

1. Ultraviolet and visible spectrophotometry,
2. Fluorescence and phosphorescence spectrophotometry,
3. Atomic spectrophotometry (emission & absorption),
4. Infra-red spectrophotometry,
5. Raman spectroscopy,
6. X-ray spectroscopy,
7. Radio chemical techniques including activation analysis,
8. NMR spectroscopy,
9. ESR spectroscopy.

b) Electeochemical Techniques

1. Potentiometry,2.Voltametry,3.Voltammetric techniques, 4.Stripping techniques, 5.Amperometric techniques, 6.Coulometry, 7.Electrogravimetry, 8.Conductance techniques.

c) Chromatographic Methods

1. Gas chromatography (GC), 2) High performance liquid chromatography (HPLC),
3) High-performance thin layer chromatography (HPTLC).

d) Miscellaneous Techniques :Thermal analysis, Mass spectrometry, Kinetic techniques.

e) Hyphenated Methods:

1. GC-MS,2)lC-MS,3)GC-IR, 4)MS-MS.

UV VISIBLE ABSORPTION SPECTROSCOPY:

The ultraviolet–visible spectrophotometer is one of the most frequently employed techniques in pharmaceutical analysis. It involves the measurements of the amount of ultraviolet (190-380 nm) or visible (380-800 nm) radiation absorbed by a substance in solution. Instruments which measure the ratio or a function of the ratio, of the intensity of two beams of light in the ultraviolet-visible region are called ultraviolet-visible spectrophotometers. Absorption of light in both the ultraviolet and visible regions of the electromagnetic spectrum occurs when the energy of the light matches that required to induce in the molecusle an electronic transition and its associated vibrational and rotational transitions.

To show absorption in UV- Vis region, molecules must contain some functional groups which absorbs UV- Vis light called as ‘chromospheres’.

The quantitative analysis by UV Visible spectrophotometry is governed by the Beer-Lambert’s law; it states that, the intensity of beam of monochromatic light when passed through transparent medium decreases exponentially as the thickness and concentration of absorbing media increases arithmetically.

$$A = \log \frac{Io}{I\text{-}t} = abc$$

Where,

A = Absorbance of the solution at particular wavelength of the light beam

I_o = Intensity of incident light beam

I_t = Intensity of transmitted light beam

a = Absorptivity of molecule at the wavelength of beam

b = Path length of cell in cm

c = Concentration of solution in gm/lit.

Terms Used in Absorption Spectroscopy:

- **Transmittance (T):** It is the ratio of intensity of transmitted light to that of incident light.

$$T = It/Io$$

- **Absorbance:** It is the negative logarithm of transmittance to the base ten.

$$A = - \log_{10} T = \log_{10} Io/It$$

$$A = abc$$

Absorptivity (a) is given by,$a = A / bc$

Where,

A = Absorbance,

b = Path length in cm.

c = Concentration in g/lit.

Molar Absorptive (ε): When concentration 'c' in equation A = abc is expressed in mole/lit. And cell length in centimeter, then absorptive is called as molar absorptive.

$\varepsilon = A / bc$

Instrumentation of Ultraviolet Spectroscopy:

The essential components of a UV spectrophotometer includes following:

1. A suitable source of radiant energy
2. A system consisting of lenses, mirror, slits, etc. which collimates and focus the beam on the sample.
3. A monochromatic system.
4. A sample holder
5. A detector system of collecting transmitted radiation
6. A suitable amplifier or read out galvanometer.

HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

High performance liquid chromatography (HPLC) is the most versatile and widely used analytical technique. It utilizes a liquid mobile phase to separate the components of mixture. These components (or analytes) are first dissolved in a solvent, and then forced to flow through a chromatographic column under high pressure. In the column, the mixture is resolved into its components. The interaction of solute with mobile and stationary phases can be manipulated through different choices of both solvent and stationary phases. As a result, HPLC acquires

a high degree of versatility not found in other chromatographic systems and it has the ability to separate a wide variety of chemical mixtures.

HPLC uses high pressure to force solvent through closed columns containing very fine particles that gives high resolution separations. The technique is used to separate and determine species in a variety of organic, inorganic, and biological materials.

The basic components of an HPLC system include a solvent reservoir, pump, injector, analytical column, detector, recorder and waste reservoir .other important elements are an inlet solvent filter, post-pump inline filter, sample filter, guard column, back pressure regulator and solvent sparging system.

The pump keeps liquid flow rate constant. Sample is injected into the injector; it is carried into the column and components are separated. Each component elutes from column one by one and is detected.

HPLC is used either in the liquid-solid adsorption chromatography mode or the liquid-liquid partition chromatography mode, either normal or reversed phase. Both partition and adsorption chromatography operates on differences in solute polarity since polarity is important in determining both adsorption and solubility. As a general rule, highly polar materials are best separated using partition chromatography, while very non polar are separated using adsorption chromatography.

Most commonly used methods in HPLC :

1. Normal phase chromatography

Mechanism: Retention by interaction of the stationary phase's polar surface with polar parts of the sample molecules.

Stationary phase: It is a bonded siloxane with polar functional group like SiO_2, Al_2O_3, $-NH_2$, -CN, $-NO_2$, - Diol.

Mobile phase: Nonpolar solvents like heptane, hexane, cyclohexane, chloroform, ethyl ether, dioxane.

Application: Separation of nonionic, non polar to medium polar substances.

Sample elution Order: Least polar components are eluted first.

2.Reverse phase chromatography

Mechanism: Retention by interaction of the stationary phase's non polar hydrocarbon chain with non polar parts of sample molecules.

Stationary phase: It is bonded siloxane with non polar functional groups like n-Octadecyl (C_{18}) or n- octyl (C_8), ethyl, phenyl, $-(CH_2)$ n-diol, $-(CH_2)$n-CN.

Mobile Phase: Polar solvents like methanol, acetonitrile, water or buffer (Sometimes with additives of THF or dioxane)

Application: Separation of nonionic and ion forming non polar to medium polar substances (carboxylic acids, hydrocarbons).

Sample elution order: Most polar components are eluted first.

Introduction of the Instrumentation:

1. Extremely precise gradient mixers (optional).
2. HPLC high pressure pumps with very constant flow.
3. Unique high accuracy, low dispersion, HPLC sample valves.

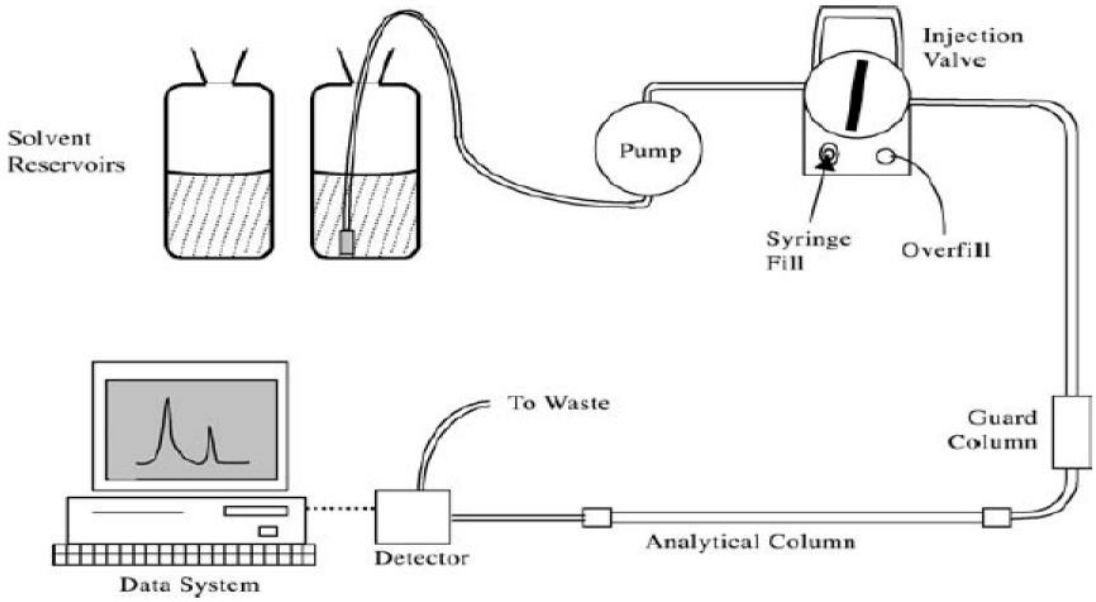
4. Very high efficiency HPLC columns with inert packing materials.

5. High sensitivity low dispersion HPLC detectors.

6. High-speed data acquisition systems.

7. Low dispersion connecting tubes for valve to column and column to detector

Schematic diagram of an HPLC instrument:



Force degradation studies:

The purpose of stability testing is to obtain information for shelf life of the medicinal product and recommend storage condition. the purpose stability studies is to ascertain how the quality of medicinal product varies as a function of time and under the influence of variety of environment factor.

Stress test :

1) Acid and alkali hydrolysis
2) Oxidative Degradation
3) Photolytic degradation
4) Heat degradation

Aim and Objective:

Aim :

The main aim of the work is to develop Simple, Accurate, precise, economical analytical method of drugs and marketed formulation by using HPLC.

Objectives:

The objectives of this dissertation work are as follows:

1) To develop simple, rapid and robust analytical method by RP-HPLC for determination of given bulk drug's and marketed formulation.

2) To validated the developed methods as per ICH Guidelines.

3) To conduct forced degradation study under various stress condition like hydrolysis (acid,alkali) oxidation, Thermal,photolysis.

4) Qualitative analysis of forced degradation products by LC-MS/MS

Literature Review:

There are many methods available for the analysis of drug acting as antihypertensive agent. Those include both classical and instrumental methods. But more popular methods are:

1) High Performance Liquid Chromatography
2) UV-Visible Spectroscopy
3) Thin layer Chromatography

Many methods have been developed & Validated for Active drug & estimation of Active drug in their formulation form, using HPLC, UPLC, UV etc.

1.Dhabale P.N, et al. (2009) suggested Development and Statistical Validation of UV Spectrophotometric Method for Estimation of Amlodipine Besylate inTablet Dosage Form by area under curve method using Wavelength range: 232- 242 nm, Solvent: Methanol over a range of 5- 25 μg/ml.

2.Patil P.R, et al. (2009) developed spectrophotometric method for simultaneous estimation of Ramipril and Amlodipine. For this, simultaneous equation method is used. The method involved measurement of absorbance at two wavelengths, 210 nm and 238 nm, λmax of Ramipril andAmlodipine respectively for concentration range of 15- 35 μg/ mL and 5- 25 μg/ mL for Ramipril and Amlodipine respectively.

3. Mehulkumar P, et al. (2009) Developed method for Simultaneous Spectroscopic Estimation of Amlodipine Besylate and Olmesartan Medoximil by derivative spectroscopic method in Tablet Dosage Form where ZCP: 239 nm of AMLO and 255 nm of OLME, Solvent: Methanol: water (1:4),Range: 2.5- 30 μg/ml of AMLO and 4- 32 μg/ml of OLME was taken as a method parameter.

4. Kamble N, et al. (2004) developed method for determination and validation of second derivative UV-spectrophotometric method for simultaneous determination of lisinopril and amlodipine from tablet dosage form using ZCP: 256 nm of AMLO and 216 nm of LIS, Methanol is used as solvent over range of 5- 30 μg/ml of AMLO and 10-60 μg/ml of LIS. Singhvi I, et al. (1998) developed Visible spectrophotometric methods for estimation of amlodipine besylate form tablets using extractive colorimetry method which involve formation complex with Bromocresol green (BCG), Bromophenyl blue (BPB) and Methylene blue

(MB),λmax: 409 nm for BCG and BPB, 668.2 nm for MB Solvent: chloroform over a range of 10-80 µg/ml. Ravi S Sankar, et al. (1997) developed RP-HPLC methods for simultaneous estimation of Amlodipine besilate and atenolol in tablet by using Phosphate buffer (pH 5.5) : Acetonitrile (50:50) as a mobile phaseand column used spherisorbC8, 250 x 3.9 mm id over a Range of 1-5 µg/ml for Amlodipine, 10- 50 µg/ml for Atenolol. Detection was done at 225 nm.

5.Fernandesa N, et al. (2008) developed UV spectroscopic methods for the simultaneous estimation of atenolol and indapamide in their combined dosage form. First method employs formation and solving of simultaneous equation using 246.4 nm and 266 nm as two wavelengths for formation of simultaneous equations. Second method was dual wavelength method, in which two wavelengths were selected for each drug in a way so that the difference in absorbance is zero for another drug. Atenolol has equal absorbance at 246.4 nm and 254.2 nm, where the differences in absorbance were measured for the determination of indapamide; similarly differences in absorbance at 266 nm and 270.2 nm were measured for the determination of atenolol. These methods obey Beer's law in the concentration range 100 to 350 and 5 to 17.5 µg/mL for atenolol and indapamide, respectively.

6. Legorburu MJ, et al. (1999) developed HPLC method with amperometric detection for the determination of the diuretic indapamide using a µBondapak C18 column is developed. The mobile phase consists of an acetonitrile–water mixture (45:55, 5mM) in KH2PO4–K2HPO4 (pH 4.0). The compound is monitored at +1200 mV with an amperometric detector equipped with a glassy carbon working electrode. Percentages of recovery are 88.3 ± 5.6 and 82.9 ±7.8 for liquid–liquid and solid–liquid extraction, respectively over the concentration range from 25 to 315 ng/mL with a reproducibility in terms of relative standard deviation of 4% for a concentration level of 0.5 µg/mL and a quantitation limit of 1mg/mL.

7. Harlikar J N, et al. (2003) developed HPLC method for simultaneous determination of Indapamide, Perindopril, Ramipril and Trandolapril from bulk drug and pharmaceutical formulation using a mobile phase consisting of 0.05M ammonium acetate (pH 2.5) and acetonitrile in volume ratio of 70:30 at a flow rate of 1.0 ml/ minute. A supelco C-18, (3, 33 x 4.6 mm) column was used as stationary phase. Quantitation was performed using UV detector at 215 nm.

8. Stefan RI, et al. (2002) two types of flow systems were selected for the simultaneous assay of S- and R-perindopril (pdp): flow injection analysis (FIA) and sequential injection analysis (SIA). The SIA system was more efficient, because of the highest precision and accuracy, and the lower consumption of sample and buffer. The amperometric biosensors used as detectors in the flow systems were based on L- and D-amino acid oxidase (AAOD). The linear concentration ranges are in the nmol l−1 range, from 120 pmol l−1 to 40 nmol l−1 (3 × S.D.), with very low detection limits.

9. Kondo T, et al. (2007) has developed method for characterization of conjugated metabolites of a new angiotensin II receptor antagonist, candesartan cilexetil, in rats by liquid chromatography/electrospray tandem mass spectrometry following chemical derivatization. In this method column C18 used for separation. The chromatographic parameters as mobile phase combination reported as Acetonitrile and water (0.05% triethylamine and 0.05% acetic acid), also calibration curve range reported as 5–500 ng/ ml.
10. Himabindu V, et al. (2007) has developed Stability-Indicating LC Method for Candesartan Cilexetil by using CN column (250 mm × 4.6 mm), 5 μm, mobile phase Phosphate buffer (pH 3.0): Acetonitrile (50:50) with detection wavelength 210 nm. iaoshan Z, et al. (2002) determined the content of candesartan cilexetil by using HPLC. The ODS column was used and mobile phase content Acetonitrile: Phosphate buffer (pH- 3.2) (80:20). The detection wavelength was 252 nm. The calibration curve range reported as 0.5 ~ 2.5 μg.

11. Luo J, et al. (2004) determined candesartan cilexetil and its related substances by using HPLC. The mobile phase was prepared as KH2PO4 mixing with triethylamine (pH -5.8) : Acetonitrile (48:52) and C18 column used with flow rate 1.0 ml/min. The wavelength was selected as 210 nm.
Chitlange SS, et al. (2008) developed Reverse Phase High Performance Liquid Chromatographic (RP-HPLC) method for simultaneous estimation of Amlodipine besylate (AMLB) and Valsartan (VAT) on RP C-18 Column (Kromasil, 250 x 4.6 mm) using acetonitrile: phosphate buffer (0.02M, pH 3.0), (56:44 v/v) as mobile phase at a flow rate of 1.0 ml/min and the detection wavelength was 234 nm. The retention time for AMLB and VAT was found to be 3.07 and 6.20 min, respectively.

PLAN OF WORK:

1) Literature survey

2) Review of the literature for given drug.

3) Development of method by HPLC.

- Selection of solvents to be used as diluents.
- Selection of mobile phase.
- Selection of wavelength by critical examination of the UV absorbance spectra of the drugs.
- Selection of column.
- Study of structure of drugs, physicochemical properties to select chromatographic parameters of the drugs.
- Selection of method for quantitative chromatographic analysis. Determination of working standards concentration range.

4) Validation of the developed method following ICH Guidelines.

- Performing recovery studies for statistical evaluation of formulation analysis.
- Performing linearity, specificity, and accuracy, precision.

5) Performing force degradation studies.

6) Compilation of data.

Instruments use in practical performance:

HPLC,UV-Spectroscopy, HPLC column , pH meter, Analytical balance, LCMS.

Probably outcome:

Present research work will involves development and validation f analytical method i.e. Stability indicating RP-HPLC method.

Thus develop will provide scientific data base for further development of sound research with sophisticated instrumentation and technique.

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